



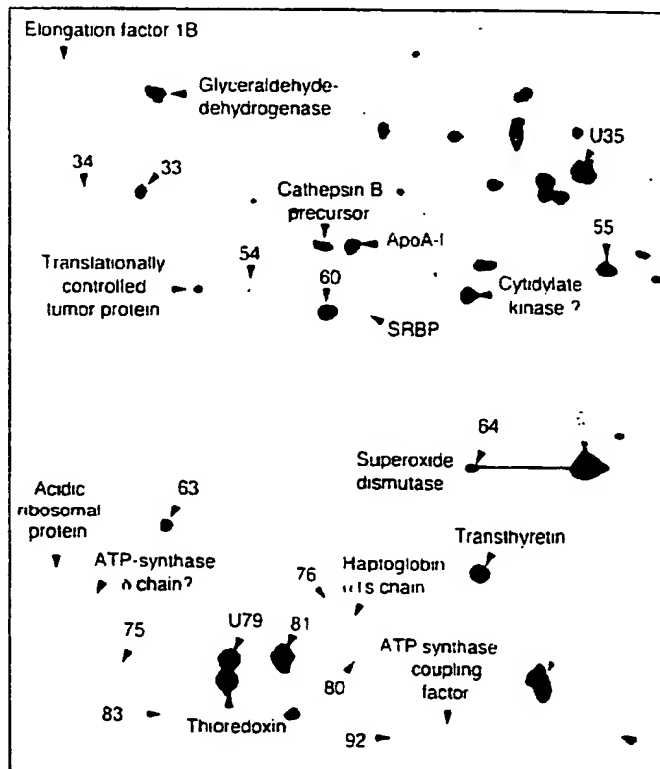
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : G01N 33/574, 33/561, C12Q 1/68	A2	(11) International Publication Number: WO 94/12881 (43) International Publication Date: 9 June 1994 (09.06.94)
<p>(21) International Application Number: PCT/EP93/03314</p> <p>(22) International Filing Date: 26 November 1993 (26.11.93)</p> <p>(30) Priority Data: 07/990,302 2 December 1992 (02.12.92) US</p> <p>(71)(72) Applicants and Inventors: HOCHSTRASSER, Denis, F. [CH/CH]; 27, chemin de la Savonnière, CH-1245 Collonge-Bellerive (CH). HUGHES, Graham, J. [GB/CH]; 12, rue du Collombier, CH-1202 Genève (CH).</p> <p>(72) Inventor; and</p> <p>(75) Inventor/Applicant (for US only): APPEL, Ron, D. [CH/CH]; 26, route des Carres, CH-1252 Meinier (CH).</p> <p>(74) Agent: BARO, Alain; Katzarov S.A., 19, rue des Epinettes, CH-1227 Genève (CH).</p>	<p>(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>	

(54) Title: A METHOD FOR DETECTING GROWING CELLS USING TRANSLATIONALLY CONTROLLED TUMOR PROTEIN p21

(57) Abstract

This invention relates to a novel method for detecting growing cells including cancer cells based upon elevated expression of a gene designated Translationally Controlled Tumor Protein p21. The invention provides for direct and indirect methods of quantifying this marker including immunoassays and nucleic acid hybridization assays.



BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

5 A METHOD FOR DETECTING GROWING CELLS USING
 TRANSLATIONALLY CONTROLLED TUMOR PROTEIN p21

 BACKGROUND OF THE INVENTION

10 This invention relates to a novel method for
detecting growing cells including cancer cells based upon
elevated expression of a gene designated translationally
controlled tumor protein p21. The invention provides for
direct and indirect methods of quantifying this marker
including immunoassays and nucleic acid hybridization assays.

15

 SUMMARY OF THE INVENTION

 This invention provides for methods of detecting a
cancerous condition in a human comprising: (a) removing a
clinical sample from the human; (b) detecting the level of
20 translationally controlled tumor protein p21 (TCTPp21) in the
sample; and,
(c) determining if the level of TCTPp21 is greater than the
level of TCTPp21 in a normal sample. The method is useful in
cancer in general and including the following cancers: breast
25 cancer, lung, ovarian cancer, cervical cancer, prostate
cancer and colon cancer. Preferred direct detection methods
are described herein but include electrophoresis procedures
and immunoassays such as Western blot assays, enzyme-linked
immunoassays (ELISA), fluorescent immunoassays,
30 radioimmunoassays and the like. Indirect methods include
using a nucleic acid hybridization assay; such assays would
include Northern assay format and the polymerase chain
reaction technology to detect specific mRNA.

35 The same methods apply to non-cancerous and actively
growing cells.

 The invention further provides for kits which detect
growing cells. These kits comprise at a minimum a container
containing a TCTPp21 binding analyte for detecting the level

of TCTPp21 in a tissue sample and a container containing a known amount of a TCTPp21 specific ligand. The binding analytes would include antibodies specific to TCTPp21 and the TCTPp21 specific ligands are typically TCTPp21 or fragments thereof or nucleic acids which bind to mRNA encoding TCTPp21.

Abbreviations and Definitions

Abbreviations used:

PDA: piperazine-diacrylamide; PVDF: polyvinylidene difluoride; SDS: sodium dodecyl sulfate; 2D-PAGE: two-dimensional polyacrylamide gel electrophoresis; CAPG: carrier ampholyte pH gradients; IPG: immobilized pH gradients; HPLC: high pressure liquid chromatography.

Definitions:

"Cancerous conditions" refers to a disease defined by unregulated cell growth in a mammalian host.

"TCTPp21 binding analyte" refers to a reagent which binds specifically to TCTPp21 or to a nucleic acid encoding TCTPp21. Such reagents would include an antibody, either a polyclonal mixture or monoclonal antibody, which binds specifically to TCTPp21 or to a cDNA which will hybridize to nucleic acid encoding TCTPp21 or portions thereof.

"TCTPp21 specific ligand" refers to a reagent which binds to a TCTPp21 binding analyte and would include TCTPp21 either native or recombinantly produced and nucleic acid encoding TCTPp21 or portions thereof.

BRIEF DESCRIPTION OF THE FIGURES

30

Figure 1:

Figure 1 shows a silver stained human liver protein 2D-PAGE picture obtained with non linear immobilized pH gradient (IPG) from 3.5 to 10 for the first dimensional separation.

35

Figure 2:

Figure 2 shows a computer match between silver stained 2D-PAGE images of liver (figure 1), plasma, red blood cell, rectal adenocarcinoma samples and an amido black stained PVDF membrane image of liver sample (figure 5). This figure was done using allspots, allareas, viewmod, modified automatch, showpairs, metal, gelsharper and showgroups programs of the Melanie/Elsie computer system [references Nos. 68, 71]. The vectors link a few matched spots between the liver "master" picture, the PVDF membrane and the other type of samples. TCTP means translationally controlled tumor protein.

Figure 3:

Figure 3 shows an enlargement of the acidic and lower molecular weight area of figure 1. "U" means unknown sequence in Swiss-Prot database. The numbers represent sequenced peptides or peptides which were attempted to be sequenced. some polypeptides could not be N-terminally microsequenced either because of too low protein concentration or because of N-terminal blockage. SRBP means serum retinol binding protein.

Figure 4:

Figure 4 shows an enlargement of the basic area of figure 1. "U" means unknown sequence in Swiss-Prot database. The numbers again identify spots which were the subject of peptide sequencing.

Figure 5:

Figure 5 shows one of the 5 to 9 amidoblack stained PVDF membranes which have been used to microsequenced 102 spots. The numbers provide a reference to figures 2-3. TCTPp21 is found at number 53.

The same membrane picture is also shown on figure 2.

DETAILED DESCRIPTION

This invention provides novel methods for detecting cell growth in humans and other mammals. The method relies upon the discovery that a previously known gene produces a protein which is specific for dividing cells. As such the presence and quantity of the protein is useful as a marker for detecting normal growth and cancerous growths.

TCTPp21 DNA and mRNA has been studied and cloned in plants (75), mouse erythroleukemia (73), mouse ascitic tumor (72) and human mammary carcinoma (74). The cDNA sequence coding for TCTP was obtained by screening a cDNA library constructed in lambda gt10 from human mammary carcinoma. The sequence is provided in Seq. ID No. 1.

Probes derived from cDNA of the translationally controlled, growth-related mouse tumor protein p23, were used to detect the clone. TCTPp21 has no known function, but its high degree of homology from plants to man underlines its probable crucial role in cell function. Previous studies showed that mouse TCTP protein concentration increased with mouse tumor growth and that its production was translationally controlled and mouse serum dependent.

A. Obtaining Clinical Samples

TCTPp21 is elevated in growing cells. The cells can be collected by conventional surgical means. A surgeon will remove tissue samples or fluid samples from a patient. The samples are suspected of containing TCTPp21 in elevated levels. Said elevated levels are indicative of active cell growth which in the case of a cancer is unregulated. Where cell death is suspected due to chemotherapy or to a natural process intrinsic to active growth of cells, TCTPp21 is expected to be present in the lymph and blood fluids of the patient.

Where tissue samples are obtained, the cells are typically lysed, then diluted in a physiologically compatible buffer such as phosphate or HEPES at a pH of between 6.5 and 7.5. The cells are lysed using a variety

of standard means which include pressure, shearing, or osmotic pressure. The whole lysate can be used in immunoassays or is optionally semi-purified to remove unwanted cellular material.

5 B. Recombinant Expression of TCTPp21.

Complementary DNA encoding TCTPp21 has been previously described by Gross, B., Gaestel, M., Bohm, H. & Bielka, H. (1989), *cDNA sequence coding for a translationally controlled human tumor protein*, Nucleic
10 Acids Res, 17(20), 8367. Using conventional recombinant technology one can obtain unlimited quantities of TCTPp21. A general review of this technology can be found in Sambrook, et al., *Molecular Cloning - A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New
15 York 1989. In brief the cDNA described by Gross et al. is recombined with an expression vector and transformed into a host cells competent to express heterologous genes. The product is then isolated and purified from the recombinant host. Such cells would include insect cells, bacterial
20 cells and mammalian cells. The choice of vector and cells are not critical and are determined by convenience and economy.

 C. Direct Methods for Detecting TCTPp21.

There are two basic methods for directly
25 measuring the presence of TCTPp21. Both methods are routine and conventional techniques. No attempt will be made to exhaustively identify all the variations possible. The two methods are electrophoretic techniques whereby the TCTPp21 is isolated from other products by its molecular
30 size and charge and immunoassays whereby the TCTPp21 is detected by its ability to be recognized by TCTPp21 specific antibodies.

Electrophoretic procedures are well known and generally described in the book, *Electrophoresis of*
35 *Proteins in polyacrylamide gels and starch gel*, Eds. Work and Work, Elsevier Science Publ. 1983. Such methods include separation of TCTPp21 from other proteins based upon its size or relative charge. The separated protein

is then detected using a variety of techniques which include colorimetric staining, radiolabels, fluorescent tagging and the like. Specific electrophoresis methods include SDS-Polyacrylamide gel electrophoresis, capillary electrophoresis, electrophoresis under nondenatured and denatured conditions.

Immunoassays are also well known and reviewed in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Pubs., N.Y. (1988). This technique relies upon the production of antibodies specific to TCTPp21. The antibodies can be either polyclonal or monoclonal. The antigens are preferably intact TCTPp21 or peptides representing the most hydrophyllic regions.

The immunoassay formats include Western blots, ELISA, fluorescent and radioimmunoassays and the like. In brief samples suspected of containing TCTPp21 are obtained and contacted with antibodies under controlled conditions. The binding of the antibodies is then detected in a variety of standard ways.

Other direct methods for detecting TCTPp21 would include high performance liquid chromatography and reverse phase liquid chromatography.

D. Indirect Methods for Detecting TCTPp21.

TCTPp21 can be detected using indirect means whereby levels of mRNA are measured. Such procedures rely upon the hybridization of complementary nucleic acids. A general review of this technology can be found in *Nucleic Acid Hybridization a Practical Approach*, Eds. B.D. Hames and S.J. Higgins, IRL Press, Washington D.C. USA, 1989. In general, the cells in a sample are lysed and the mRNA of a tissue sample is isolated using a conventional technique such as with a polyoligo dT column. The isolated mRNA is then assayed using probes which could include the entire cDNA sequence for TCTPp21 mRNA as provided in Seq. ID No. 1 or specific fragments thereof. Other operable methods would include isolation of whole nucleic acid and detection of mRNA in the mixture.

A basic technique for detecting mRNA is the Northern blotting technique where mRNA is separated according to size in an electrophoretic matrix. The mRNA is then transferred to a solid support and labelled cDNA probes are used to detect the presence of specific mRNA.

A second means to identify specific mRNA in a cell lysate involves the use of the polymerase chain reaction. A general review of this technology is found in PCR Protocols A Guide to Methods and Applications, Eds., Innis, Gelfand, Sninsky and White, Academic Press, Inc. 1990. Chapter 8 entitled "Competitive PCR for Quantitation of mRNA" describes this technology in detail.

E. Kits

This invention further provides for kits. Such kits would include materials and instructions for detecting TCTPp21 either directly or indirectly as described above. The kits would contain reagents such as recombinant TCTPp21, TCTPp21 specific antibodies and miscellaneous reagents for controls, PCR primers and TCTPp21 specific nucleic acid probes, detection reagents such as enzyme/substrate combinations, fluorescent labels, solid supports for antibodies or nucleic acids and the like. The specific combination of reagents are determined by the assay being presented by the kit.

Examples

The examples provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters which could be changed or modified to yield essentially similar results.

Example 1. The use of 2D electrophoresis mapping to identify TCTPp21 as a new cancer marker.

The following materials and methods were used to determine that the expression levels of TCTPp21 were elevated in growing cells:

Acrylamide, piperazine diacrylamide, tetramethyl-ethylenediamine, ammonium persulfate, glycine, sodium

dodecylsulfate were from Bio-Rad (Glattbrugg, Switzerland); PVDF membranes were from Millipore (Bedford, MA, USA) or Bio-Rad; tris(hydroxymethyl)-aminomethane (hydrochloride), Nonidet P-40[®],
5 chloramidopropyl-dimethyl-hydroxypropane-sulfonate were from Sigma (Deisenhofen, Germany); citric acid, urea and dithiothreitol were purchased from Merck (Darmstadt, Germany) and ResolytesTM 4-8 from Chemie
10 Brunschwig (Basel, Switzerland); Immobiline[®] were from Pharmacia-LKB (Uppsala, Sweden).

In the early course of this work, 2D-PAGE using carrier ampholytes in the first dimension was performed as described previously [44, 45]. The isoelectrophoretic separation was performed in a model 175 chamber with a model
15 3000/300xi power supply from Bio-Rad. For SDS-PAGE, the Protean IITM chamber and casting chamber (160 x 200 x 1.5 mm gels) were used. The power supply (700V, 1.6 A) was from Hewlett-Packard (Palo Alto, CA). The gradient pourer was the Bio-Rad model 395.

20 More recently, the isoelectrophoretic separation (immobilized pH gradients) was performed in a MultiphoreTM apparatus with a 5000 V power supply from Pharmacia/LKB (Broma, Sweden). Preparative isoelectrofocusing was also done using narrow immobilized pH gradients and the equipment
25 mentioned above. In later experiments, the gradient pourer was the computerized gradient pourer AngeliqueTM (LargeScaleBiology, Rockville, MD). N-terminal sequence determinations were performed using either a model 473A or 477A microsequencer (Applied Biosystems, Foster City, CA)
30 equipped with ProblottTM reaction cartridges.

Plasma and red blood cell sample collection and preparation were done according to previous publications [44, 46-48]. The liver and other biopsy samples were collected in the operating room at the time of surgery and
35 immediately frozen in liquid nitrogen. They were prepared as follows.

Five frozen slices (20µm x 5 mm x 10 mm) of liver biopsy were mixed in an Eppendorf[®] tube with 300 µL of a

solution containing 8 M Urea, 4 % w/v CHAPS, 40 mM Tris, 10 mM DTE and a trace of Bromophenol Blue. Sixty μ L of the final diluted liver sample were loaded on each IPG gel strip.

5 For micropreparative gel sample load the sample were treated as follows. Twenty frozen slices (20 μ m x 5 mm x 10 mm) of liver biopsy were placed in an Eppendorf[®] tube with 400 μ L of a solution containing 8 M Urea, 4 % w/v CHAPS, 40 mM Tris, 10 mM DTE and a trace of Bromophenol
10 Blue. Ninety μ L of the final diluted liver sample were loaded on each IPG gel strip.

In addition, rectal cancer, lung cancer, normal liver and brain tissue samples were prepared according to the above protocol.

15

A. Two-dimensional polyacrylamide gel electrophoresis

Two-dimensional gel electrophoresis was performed essentially as described previously for carrier ampholyte pH gradient separation [44, 45] and as described by Goerg et
20 al. [49] for immobilized pH gradients. Running conditions for the first dimensional separation were as follows: voltage was linearly increased from 300 V to 3500 V during the first 3 hours; the strips were allowed to run at 3500 V for 3 additional hours and the voltage was then increased to
25 5000 V to give a total of 100 KVh for analytical gels and 450 KVh in a 4 days run for micropreparative gels. After the second dimension, the gels were stained with silver [44, 45, 50, 51]. When 2D-PAGE was followed by electroblotting and microsequencing analysis (see below), the amount of sample
30 loaded in the first dimension was multiplied by 5 as mentioned above.

B. Electroblotting, staining and microsequencing procedures

35 Electroblotting onto PVDF membranes was done essentially according to Towbin et al. [52] and Matsudaira [53]. Membranes were stained with amidoblack, destained with water and dried. Spots of interest were excised, dried under

nitrogen and kept in Eppendorf[®] tubes at -20°C until microsequencing was performed [53-57].

Routinely, ten to twelve Edman degradation cycles (see section 2.2) were performed for each spot. A search in the Swiss-Prot database [58] was made to establish identity or homology to already known proteins.

Results

C. Carrier ampholytes versus immobilized pH gradients

In the past, human liver protein maps, obtained using carrier ampholyte pH gradients for the first dimensional separation, could not be compared to plasma, red blood cell or rectal cancer tissue maps. The cathodic drift, seen with carrier ampholyte pH gradients, altered the focusing of basic polypeptides too much. Furthermore, proteins as well as ions present in the sample influenced the pH gradient. Consequently, the pH gradient varied from sample to sample. Immobilized pH gradients circumvented this problem and provided much more reproducible patterns. It was thus possible to localize proteins on the immobilized pH gradient 2D-PAGE pictures by direct comparison of different sample separations (liver, rectal adenocarcinoma tissue, red blood cell and plasma) (see figures 2-4).

D. Previously identified proteins

In plasma/serum maps, more than 350 spots representing more than 40 proteins have been identified so far [25, 26, 46, 47, 59-63]. In red blood cell maps, 12 polypeptides have been identified so far [27, 48]. Figures 2 and 3 show the localisation of several plasma and red blood cell polypeptide chains on the human liver protein map (enlargements of the silver stained immobilized pH gradient 2D-PAGE image of figure 1). The identification was done by overlapping liver, red blood cell and plasma protein maps. These proteins could be either trapped in the liver blood vessels or localized in the bile ducts, portal area or hepatocytes.

E. Newly identified polypeptide spots

A group of more than 3000 spots, probably representing more than 2500 polypeptide chains, are readily detected by the Melanie computer system [64-68] on silver stained 2D-PAGE image of liver biopsy samples. In order to build our knowledge of the human liver protein map (and other tissues as well), to provide further information in disease associated protein pattern changes and to discover new liver proteins, we have attempted to N-terminally microsequence 102 spots transblotted onto PVDF membranes. A maximum of 9 2D-PAGE were run for any unique sequence determination. We did not obtain any sequencing signal for 43% of the spots either because of too low a protein concentration or because of N-terminal blockage. In one case, a double sequencing signal was obtained, not providing any possible interpretation (spot number 50, figures 4 & 5). Two spots (2%) gave double sequencing signals providing the identification of two polypeptides (spot numbers 71 and 98, figures 4 & 5). For 11 % of the spots, the sequence was unequivocal, but no matches could be found in the Swiss-Prot database. Those were considered as unknown proteins. 44% of the spots were positively identified as known proteins or derived fragments.

Figure 5 shows an amidoblack stained PVDF membrane picture. The red arrows highlight the spots which have been successfully N-terminally microsequenced. The blue arrows highlight the spots which have not been successfully N-terminally microsequenced. The numbers provide references to table 1. Spot number 53 was found to be the translationally controlled tumor protein (spot number 53, figures 2,3 & 5).

Based on this discovery, we did further study. The liver piece used in this study was obtained from a patient who suffered a neuroendocrine tumor. The tumor originated in the pancreas and metastasized in the liver. The patient underwent successfully a right hemi-hepatectomy for tumor mass reduction. We microsequenced a 21 Kd acidic spot which was found to be the translationally controlled tumor protein (spot number 53, figures 2,3 & 5). This

protein spot was then found also on rectal adenocarcinoma biopsy (figure 2), on lung adenocarcinoma and on hepatocarcinoma derived cell line patterns, the latter having a pattern very similar to liver biopsies. The TCTP
5 spot was not found in normal liver and brain maps.

Example 2. An immunoassay to detect cancer in a human patient.

A patient suspected of hosting cancerous liver cells is
10 biopsied using conventional surgical techniques. The liver tissue is homogenized and the cell lysate is diluted with a physiological buffer. A plastic ELISA titration plate having a first section of holes previously treated with TCTPp21 specific antibodies is pre-prepared. A second
15 section of the plate provides control antibodies which do not bind to TCTPp21. A portion of both sections is contacted with various dilutions of the sample and with a control solution containing TCTPp21. The plates are incubated and then washed. The bound TCTPp21 is then
20 detected using TCTPp21 specific antibodies bearing a fluorescent or enzyme label.

Example 3. A nucleic acid hybridization assay to detect cancer in a human patient.

25 The sample from the patient described in example 2 is homogenized and the total mRNA extracted using conventional mRNA purification technology which takes advantage of the unique polyA tails of mRNA. The mRNA is then assayed using the Northern blotting techniques taught in Sambrook using a
30 cDNA probe derived from the sequence provided herein encoding TCTPp21.

References

(25) Anderson, N. L. & Anderson, N. G., Proc. Natl. Acad. Sci. U.S.A 1977, 74, 5421-5425.
35

(26) Anderson, N. L., & Anderson, N. G., Electrophoresis 1991, 12, 883-906.

(27) Edwards, J. J., Anderson, N. G., Nance, S. L. & Anderson, N. L., Blood 1979, 53, 1121-32.

5 (44) Hochstrasser, D. F., Harrington, M. G., Hochstrasser, A. C., Miller, M. J. & Merrill, C. R., Anal Biochem 1988, 173, 424-35.

(45) Hochstrasser, D. F. & Merrill, C. R., Appl Theor Electrophor 1988, 1, 35-40.

10 (46) Hochstrasser, A. C., James, R. W., Martin, B. M., Harrington, M., Hochstrasser, D., Pometta, D. & Merrill, C. R., Appl Theor Electrophor 1988, 1, 73-6.

15 (47) Hughes, G. J., Frutiger, S., Paquet, N., Ravier, F., Pasquali, C., Sanchez, J.-C., James, R., Tissot, J.-D., Bjellqvist, B. & Hochstrasser, D. F., Electrophoresis 1992, in press.

20 (48) Golaz, O., Walser, C., Hochstrasser, D. F. & Balant, L., Appl Theor Electrophor 1992, in press.

(49) Goerg, A., Postel, W. & Guenther, S., Electrophoresis 1988, 9, 531-46.

25 (50) Oakley, B. R., Kirsch, D. R. & Morris, N. R., Anal. Biochem. 1980, 105, 361-3.

(51) Rabilloud, T., Electrophoresis 1992, 13, 429-39.

30 (52) Towbin, H., Staehelin, T. & Gordon, J., Proc. Natl. Acad. Sci. USA 1979, 76, 4350-4.

(53) Matsudaira, P., J. Biol. Chem. 1987, 262, 10035-8.

35 (54) Eckerskorn, C., Jungblut, P., Mewes, W., Klose, J. & Lottspeich, F., Electrophoresis 1988, 9, 830-8.

- (55) Eckerskorn, C. & Lottspeich, F., Electrophoresis 1990, 11, 554-61.
- 5 (56) Jungblut, P., Eckerskorn, C., Lottspeich, F. & Klose, J., Electrophoresis 1990, 11, 581-8.
- (57) Wildenauer, D. B., Körschenhausen, D., Hoechtlen, W., Ackenheil, M., Kehl, M. & Lottspeich, F., Electrophoresis 1991, 12, 487-92.
- 10 (58) Bairoch, A. & Boeckman, B., Nucleic Acids Res 1991, 19, 2247-9.
- (59) Anderson, N. L. & Anderson, N. G., Biochem Biophys Res Commun 1979, 88, 258-65.
- 15 (60) Tracy, R. P., Currie, R. M., Kyle, R. A. & Young, D. S., Clin Chem 1982, 28, 900-7.
- (61) Tracy, R. P., Currie, R. M. & Young, D. S., Clin Chem 1982, 28, 890-9.
- 20 (62) Tracy, R. P. & Young, D. S.; in (Eds.) J. E. Celis et R. Bravo. (1984). Clinical Applications of Two-Dimensional Gel Electrophoresis. Orlando: Academic Press Inc.; 193-240.
- 25 (63) James, R. W., Hochstrasser, D., Tissot, J. D., Funk, M., Appel, R., Barja, F., Pellegrini, C., Muller, A. F. & Pometta, D., J Lipid Res 1988, 29, 1557-71.
- 30 (64) Appel, R., Hochstrasser, D., Roch, C., Funk, M., Muller, A. F. & Pellegrini, C., Electrophoresis 1988, 9, 136-42.
- 35 (65) Pun, T., Hochstrasser, D. F., Appel, R. D., Funk, M., Villars, A. V. & Pellegrini, C., Appl Theor Electrophor 1988, 1, 3-9.

- (66) Roch, C., Pun, T., Hochstrasser, D. F. & Pellegrini, C., Comput Med Imaging Graph 1989, 13, 383-91.
- 5 (67) Hochstrasser, D. F., Appel, R. D., Vargas, R., Perrier, R., Vurlod, J. F., Ravier, F., Pasquali, C., Funk, M., Pellegrini, C., Muller, A. F. & Scherrer, J.R., Md Comput 1991, 8, 85-91.
- 10 (68) Appel, R. D. & Hochstrasser, D. F., Electrophoresis 1991, 12, 722-35.
- (73) Chitpatima, S. T., Makrides, S., Bandyopadhyay, R. & Brawerman, G. (1988). Nucleotide sequence of a major
15 messenger RNA for a 21 kilodalton polypeptide that is under translational control in mouse tumor cells. Nucleic Acids Res, 16(5), 2350.
- (74) Gross, B., Gaestel, M., Bohm, H. & Bielka, H. (1989).
20 cDNA sequence coding for a translationally controlled human tumor protein. Nucleic Acids Res, 17(20), 8367.
- (75) Pay, A., Heberle-Bors, E. & Hirt, H. (1992).
25 An alfalfa cDNA encodes a protein with homology to translationally controlled human tumor protein.
Plant Molecular Biology, 19, 501-503.

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 781 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: c-DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
1  CCCCCCGAGCGCCGCTCCGGCTGCACCGCGCTCCTCCGAGTTTCAGGCTCGTGCTAAG
61  CTAGCGCCGTCGTCGTCTCCCTTCAGTCGCCATCATGATTATCTACCGGGACCTCATCAG
    M I I Y R D L I S
121 CCACGATGAGATGTTCTCCGACATCTACAAGATCCGGGAGATCCGGACGGGTTGTGCCT
    H D E M F S D I Y K I R E I A D G L C L
181 GGAGGTGGAGGGGAAGATGGTCAGTAGGACAGAAGGTAACATTGATGACTCGCTCATTGG
    E V E G K M V S R T E G N I D D S L I G
241 TGGAAATGCCTCCGCTGAAGGCCCGAGGGCGAAGGTACCGAAAGCACAGTAATCACTGG
    G N A S A E G P E G E G T E S T V I T G
301 TGTCGATATTGTCATGAACCATCACCTGCAGGAAACAAGTTTCACAAAAGAAGCCTACAA
    V D I V M N H H L Q E T S F T K E A Y K
361 GAAGTACATCAAAGATTACATGAAATCAATCAAAGGGAAACTTGAAGAACAGAGACCAGA
    K Y I K D Y M K S I K G K L E E Q R P E
421 AAGAGTAAACCTTTTATGACAGGGGCTGCAGAACAAATCAAGCACATCCTTGCTAATTT
    R V K P F M T G A A E Q I K H I L A N F
481 CAAAACTACCAGTTCTTTATTGGTGAAAACATGAATCCAGATGGCATGGTTGCTCTATT
    K N Y Q F F I G E N M N P D G M V A L L
541 GGACTACCGTGAGGATGGTGTGACCCCATATATGATTTTCTTTAAGGATGGTTTAGAAAT
    D Y R E D G V T P Y M I F F K D G L E M
601 GGAAAAATGTTAACAAATGTGGCAATTATTTTGATCTATCACCTGTCATCATAACTGGC
    E K C
661 TTCTGCTTGTCATCCACACAACACCAGGACTTAAGACAAATGGGACTGATGTCATCTTGA
721 GCTCTTCATTTATTTTGACTGTGATTTATTTGGAGTGGAGGCATTGTTTTTAAGAAAAA'
781 ATGTCATGTAGGTTGTCTAAAAATAAAATGCATTTAACTCATTGAGAGAAAAAAA
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Hochstrasser, Denis F.
Hughes, Graham J.
Appel, Ron D.
- (ii) TITLE OF INVENTION: A Method for Detecting Growing Cells
Using Translationally Controlled Tumor Protein p21
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: KATZAROV S.A.
 - (B) STREET: 19, rue des Epinettes
 - (C) CITY: Geneva
 - (D) STATE: GE
 - (E) COUNTRY: Switzerland
 - (F) ZIP: 1227
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/990,302
 - (B) FILING DATE: 02-DEC-1992
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Reverdin, André
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (+4122) 342 66 30
 - (B) TELEFAX: (+4122) 342 66 15

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 838 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 95..610

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCCCCCAG CGCCGCTCCG GCTGCACCGC GCTCGCTCCG AGTTTCAGGC TCGTGCTAAG
60

CTAGCGCCGT CGTCGTCTCC CTTAGTCGC CATC ATG ATT ATC TAC CGG GAC
112

Met Ile Ile Tyr Arg Asp
1 5

CTC ATC AGC CAC GAT GAG ATG TTC TCC GAC ATC TAC AAG ATC CGG GAG
160

Leu Ile Ser His Asp Glu Met Phe Ser Asp Ile Tyr Lys Ile Arg Glu
10 15 20

ATC GCG GAC GGG TTG TGC CTG GAG GTG GAG GGG AAG ATG GTC AGT AGG
208

Ile Ala Asp Gly Leu Cys Leu Glu Val Glu Gly Lys Met Val Ser Arg
25 30 35

ACA GAA GGT AAC ATT GAT GAC TCG CTC ATT GGT GGA AAT GCC TCC GCT
256

Thr Glu Gly Asn Ile Asp Asp Ser Leu Ile Gly Gly Asn Ala Ser Ala
40 45 50

GAA GGC CCC GAG GGC GAA GGT ACC GAA AGC ACA GTA ATC ACT GGT GTC
304

Glu Gly Pro Glu Gly Glu Gly Thr Glu Ser Thr Val Ile Thr Gly Val
55 60 65 70

GAT ATT GTC ATG AAC CAT CAC CTG CAG GAA ACA AGT TTC ACA AAA GAA
352

Asp Ile Val Met Asn His His Leu Gln Glu Thr Ser Phe Thr Lys Glu
75 80 85

GCC TAC AAG AAG TAC ATC AAA GAT TAC ATG AAA TCA ATC AAA GGG AAA
400

Ala Tyr Lys Lys Tyr Ile Lys Asp Tyr Met Lys Ser Ile Lys Gly Lys
90 95 100

CTT GAA GAA CAG AGA CCA GAA AGA GTA AAA CCT TTT ATG ACA GGG GCT
448

Leu Glu Glu Gln Arg Pro Glu Arg Val Lys Pro Phe Met Thr Gly Ala
105 110 115

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ile Ile Tyr Arg Asp Leu Ile Ser His Asp Glu Met Phe Ser Asp
 1           5           10           15
Ile Tyr Lys Ile Arg Glu Ile Ala Asp Gly Leu Cys Leu Glu Val Glu
 20           25           30
Gly Lys Met Val Ser Arg Thr Glu Gly Asn Ile Asp Asp Ser Leu Ile
 35           40           45
Gly Gly Asn Ala Ser Ala Glu Gly Pro Glu Gly Glu Gly Thr Glu Ser
 50           55           60
Thr Val Ile Thr Gly Val Asp Ile Val Met Asn His His Leu Gln Glu
 65           70           75           80
Thr Ser Phe Thr Lys Glu Ala Tyr Lys Lys Tyr Ile Lys Asp Tyr Met
 85           90           95
Lys Ser Ile Lys Gly Lys Leu Glu Glu Gln Arg Pro Glu Arg Val Lys
100           105           110
Pro Phe Met Thr Gly Ala Ala Glu Gln Ile Lys His Ile Leu Ala Asn
115           120           125
Phe Lys Asn Tyr Gln Phe Phe Ile Gly Glu Asn Met Asn Pro Asp Gly
130           135           140
Met Val Ala Leu Leu Asp Tyr Arg Glu Asp Gly Val Thr Pro Tyr Met
145           150           155           160
Ile Phe Phe Lys Asp Gly Leu Glu Met Glu Lys Cys
165           170

```

GCA GAA CAA ATC AAG CAC ATC CTT GCT AAT TTC AAA AAC TAC CAG TTC
496
Ala Glu Gln Ile Lys His Ile Leu Ala Asn Phe Lys Asn Tyr Gln Phe
120 125 130

TTT ATT GGT GAA AAC ATG AAT CCA GAT GGC ATG GTT GCT CTA TTG GAC
544
Phe Ile Gly Glu Asn Met Asn Pro Asp Gly Met Val Ala Leu Leu Asp
135 140 145 150

TAC CGT GAG GAT GGT GTG ACC CCA TAT ATG ATT TTC TTT AAG GAT GGT
592
Tyr Arg Glu Asp Gly Val Thr Pro Tyr Met Ile Phe Phe Lys Asp Gly
155 160 165

TTA GAA ATG GAA AAA TGT TAACAAATGT GGCAATTATT TTGGATCTAT
640
Leu Glu Met Glu Lys Cys
170

CACCTGTCAT CATAACTGGC TTCTGCTTGT CATCCACACA ACACCAGGAC TTAAGACAAA
700

TGGGACTGAT GTCATCTTGA GCTCTTCATT TATTTTGACT GTGATTTATT TGGAGTGGAG
760

GCATTGTTTT TAAGAAAAAC ATGTCATGTA GGTGTCTAA AAATAAAATG CATTTAAACT
820

CATTTGAGAG AAAAAAAA
838

WHAT IS CLAIMED IS:

1. A method of detecting a cancerous condition in a
5 human comprising:
 - (a) removing a clinical sample from the human;
 - (b) detecting the level of translationally controlled tumor protein p21 (TCTPp21) in the sample; and,
 - (c) determining if the level of TCTPp21 is greater than
10 the level of TCTPp21 in a normal sample.
2. A method of claim 1 wherein the cancerous condition
is selected from the group of cancers consisting of: breast
cancer, ovarian cancer, cervical cancer, prostate cancer
15 and colon cancer.
3. A method of claim 1 wherein the level of TCTPp21 is
detected using an immunoassay.
- 20 4. A method of claim 3 wherein the immunoassay is an
ELISA format.
5. A method of claim 1 wherein the level of TCTPp21 is
indirectly detected using a nucleic acid hybridization
25 assay.
6. A method of claim 1 where the TCTPp21 is detected
using electrophoresis.
- 30 7. A method of detecting growing cells in a human
comprising:
 - (a) removing a clinical sample from the human;
 - (b) detecting the level of translationally controlled tumor protein p21 (TCTPp21) in the sample; and,
 - 35 (c) determining if the level of TCTPp21 is greater than
the level of TCTPp21 in a non-growing sample.

8. A method of claim 7 wherein the level of TCTPp21 is detected using an immunoassay.

5 9. A method of claim 8 wherein the immunoassay is an ELISA format.

10 10. A method of claim 7 wherein the level of TCTPp21 is indirectly detected using a nucleic acid hybridization assay.

11. A method of claim 7 where the TCTPp21 is detected using electrophoresis.

15 12. A kit for detecting growing cells which comprises: a container containing a TCTPp21 binding analyte for detecting the level of TCTPp21 in a tissue sample and a container containing a known amount of a TCTPp21 specific ligand.

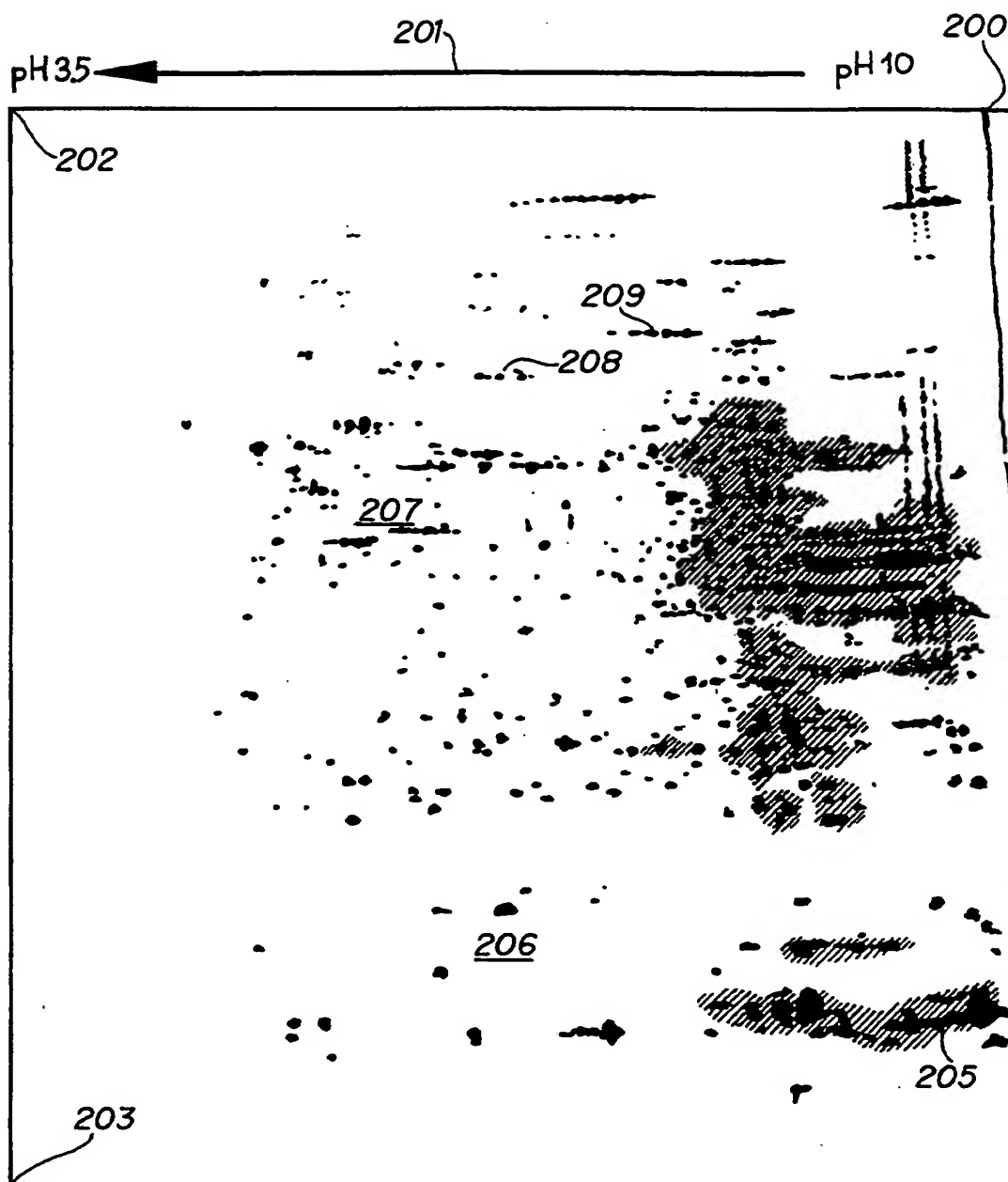
20 13. A kit of claim 12 wherein the binding analyte is an antibody specific for TCTPp21.

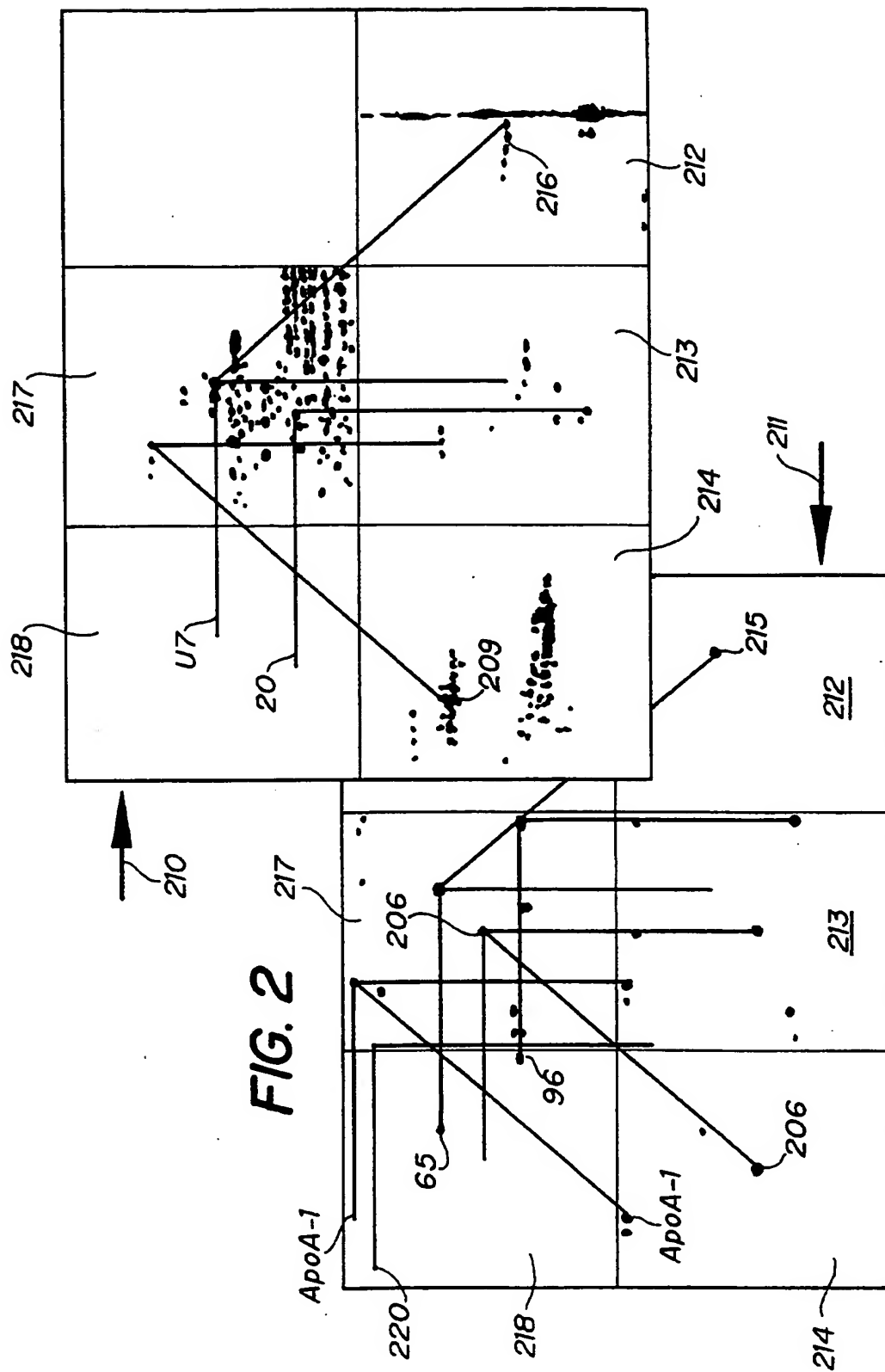
25 14. A kit of claim 12 wherein the binding analyte is a nucleic acid which hybridizes to a mRNA encoding TCTPp21.

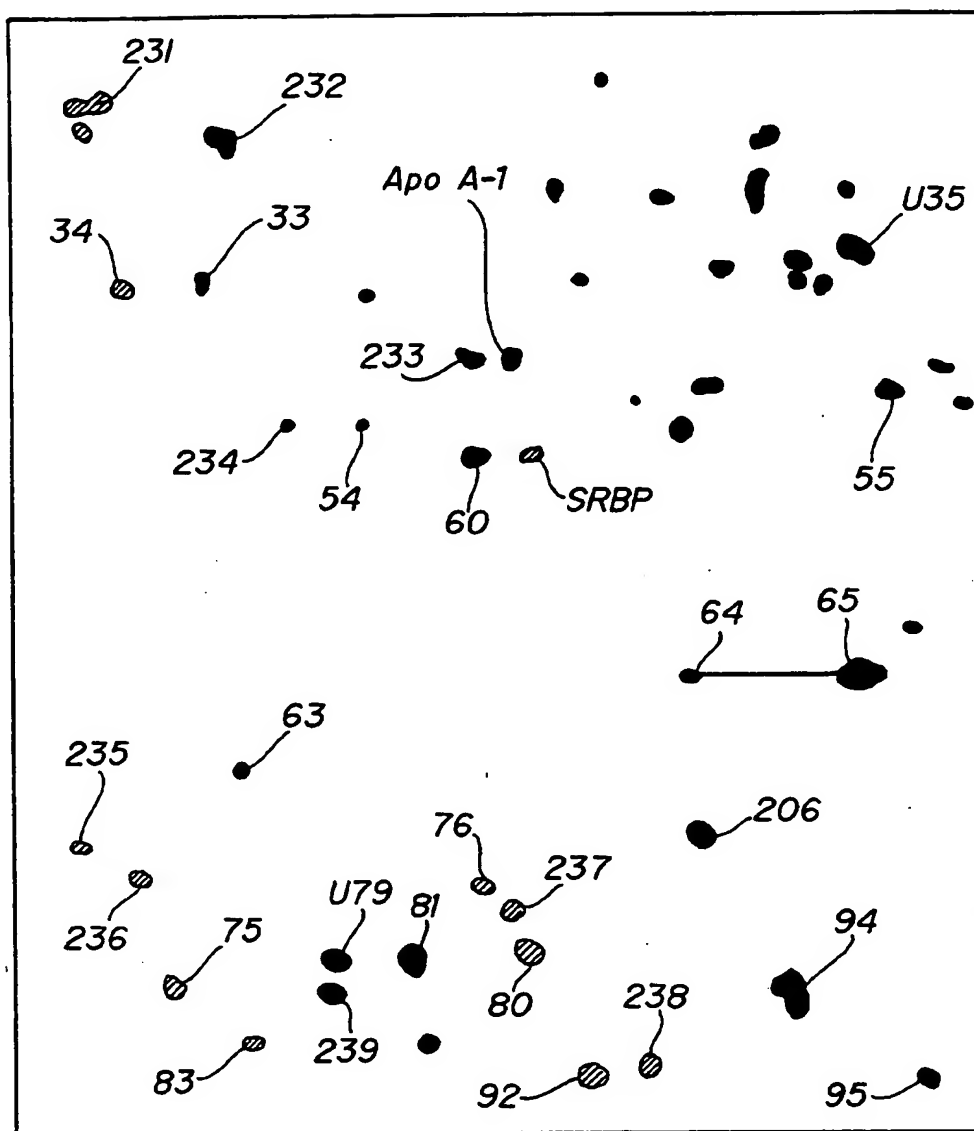
15. A kit of claim 12 wherein the TCTPp21 specific ligand is TCTPp21.

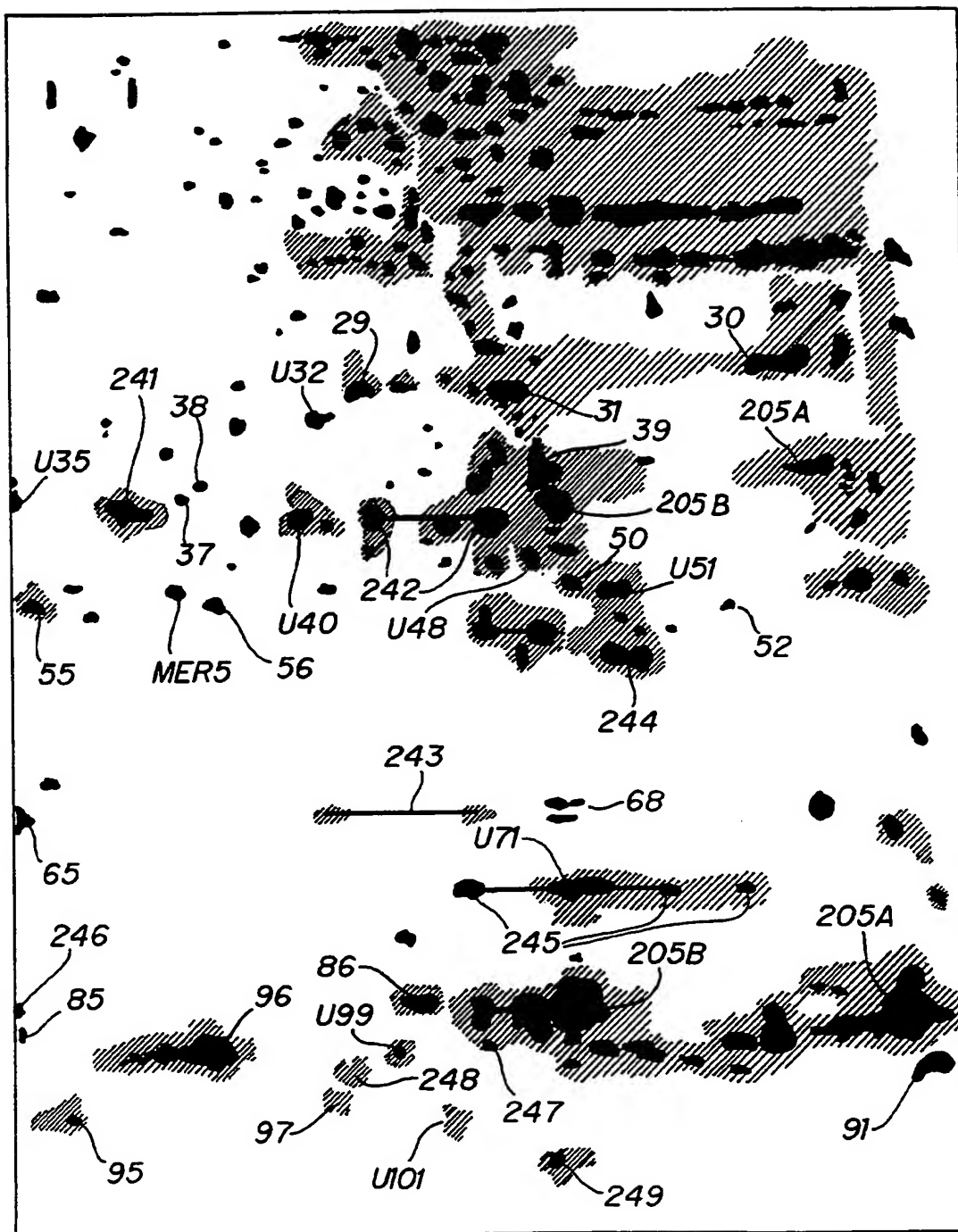
30 16. A kit of claim 12 wherein the TCTPp21 specific ligand is a nucleic acid complementary to the human cDNA encoding TCTPp21.

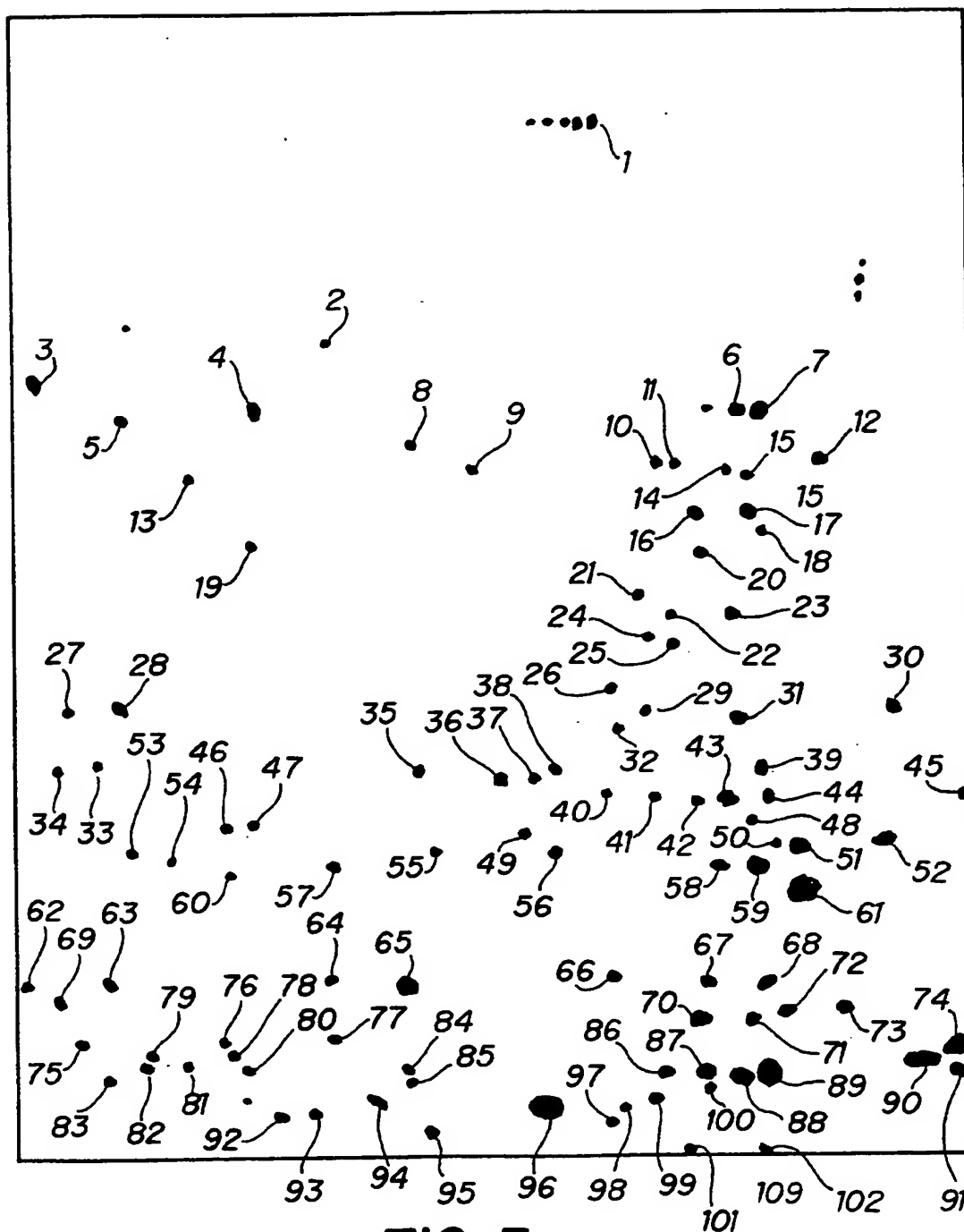
35

**FIG. 1**



**FIG. 3**

**FIG. 4**

**FIG. 5**

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.